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EP-A- 0 170 540
DE-A- 3 339 861

⑤⑥ References cited :
CHEMICAL ABSTRACTS, vol. 81, no. 15, 14th
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Description

Background of the Invention

5 The eye is fundamentally one of the most important organs during life. Because of aging, diseases and other factors which can adversely affect vision, the ability to maintain the health of the eye becomes all important. The leading cause of blindness is the inability in the treatment of eye diseases to introduce drugs or therapeutic agents into the eye. The exact mechanism or reason is not known, but certainly the blood eye barrier (as analogous to the blood brain barrier) may be an important factor. On the other hand, when a drug is
10 injected into the eye, it quickly washes out or is depleted from within the eye into the general circulation. From the therapeutic standpoint, this may be as difficult as giving no drug at all. Because of this inherent difficulty of delivering drugs into the eye, successful medical treatment of ocular diseases is totally inadequate.

The need for a solution is even more pressing in that the cause of a number of ocular diseases have now been identified and many are amenable to treatment if a proper mode of therapeutic delivery is available. It is
15 therefore of great interest to develop modes of treatment which obviate the limitations of present modes of therapy.

There has been a substantial resistance to introduce drugs directly into one or both chambers of the eye. The many uncertainties associated with the distribution of the drug, rate of release, binding to eye components, concentration by cells, rapid loss and/or inactivation, and the like, is discouraging for the efficacy of direct intro-
20 duction.

Relevant Literature

Heller (1), Biodegradable Polymers in Controlled Drug Delivery, In: CRC Critical Reviews in Therapeutic
25 Drug Carrier Systems, Vol. 1, CRC Press, Boca Raton, FL, 1987, pp 39-90, describes encapsulation for controlled drug delivery. See also, Heller (2), In: Hydrogels in Medicine and Pharmacy, N.A. Peppes ed., Vol. III, CRC Press, Boca Raton, FL, 1987, pp 137-149, describes bioerodible polymers. Heller, J. of Controlled Release (1985) 2:167-177; Leong et al., BioMaterials (1986) 7:364-371 describes polyanhydride microspheres. Jack-
anicz et al., Contraception (1973) 8:227; Yolles et al., In: Controlled Release of Biologically Active Agents, Tan-
30 quary et al. eds, Plenum Press, New York, NY, 1974, Chapter 3; Liu et al., Ophthalmology (1987) 94:1155-1159 and references cited therein report a study for the intravitreal use of liposomes for therapeutic treatment of eye disease. See also, Cutright et al., Oral Surgery, Oral Medicine, and Oral Pathology (1974) 37:142 and Shindler
et al., Contemporary Topics in Polymer Science (1977) 2:251-289. Anderson et al., Contraception (1976)
13:375 and Miller et al., J. Biomed. Materials Res. (1977) 11:711, describe various properties of poly(DL-lactic
35 acid). Patents of interest include 3,416,530; 3,626,940; 3,828,777; 3,870,791; 3,916,899; 3,944,064; 3,962,414; 4,001,388; 4,052,505; 4,057,619; 4,164,559; 4,179,497; 4,186,184; 4,190,642; 4,281,654; 4,303,637; 4,304,765; 4,304,767; 4,439,198; 4,452,776; 4,474,751; 4,613,330; and 4,617,186.

The following books describe the use of liposomes as drug carriers. Papahadjopoulos (1978) The Annals of the New York Academy of Sciences, Vol. 308, and Gregoriades and Allison (1980) Liposomes in Biological
40 Systems, John Wiley & Sons. Leserman et al., Nature (1981) 293:226-228; Barbet et al., Supramol. Struct. Cell Bio. Chem. (1981) 16:243-258; Heath et al., Science (1980) 210:539-541.

The preparation of liposomes is disclosed by Szoka and Papahadjopoulos, Proc. Natl. Acad. Sci USA (1978) 75:145:149.

SUMMARY OF THE INVENTION

The use of a composition for the manufacture of a medicament for treating an eye condition, proposed to be introduced into the anterior and/or posterior chambers of the eye to provide a therapeutically effective amount of the drug.

50 The use of a composition for the manufacture of a medicament for treating an eye condition which composition comprises :

a pharmacologically active agent surrounded by a barrier to immediate release wherein said composition is capable of introduction into a chamber of said eye and said agent is released from said barrier at a rate to provide an effective dosage of said agent over an extended period of time.

55 The said barrier is preferably selected from pharmacologically acceptable biodegradable polymers and liposomes.

Also the use of a composition for the manufacture of a medicament for treating an eye condition which composition comprises :

a pharmacologically active agent surrounded by a barrier to immediate release wherein said composition is capable of introduction into a chamber of said eye and said agent is released from said barrier at a rate to provide an effective dosage of said agent over an extended period of time.

The appended claims are included as part of the specification.

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DESCRIPTION OF THE SPECIFIC EMBODIMENTS

Ocular conditions, diseases and disorders, are treated by introducing slow release drug-containing micro-capsules directly into the anterior and/or posterior chambers of the eye. The microcapsules are formulated to include one or more drugs which may be released over an extended period of the time at a therapeutically effective dosage into the vitreous humor. In this manner, drugs released from microcapsules placed into the anterior chamber will reach the cornea, aqueous humor, trabecular mesh work, iris, lens, and related structures in the anterior chamber. Micro-capsules introduced into the posterior chamber are diffused throughout the vitreous in the chamber and into the entire retina (which consists of 10 different layers), into the choroid and the opposed sclera. Thus, the drug will be available at the site(s) where the drug is needed and will be maintained at an effective dosage, rather than rapidly being washed out or as in the case of systemic administration, requiring greatly elevated levels of drug administration to the host to achieve an effective level in the eye. Where the drugs are encapsulated in liposomes, concentrated doses of medication can be delivered into the eye for a more effective, less toxic treatment.

The primary element of the capsule will be the polymeric or lipid encapsulating agent. The compositions will be biocompatible, preferably biodegradable.

For the most part, the polymeric compositions will be organic esters or ethers, which when degraded result in physiologically acceptable degradation products, including the monomers. Anhydrides, amides, orthoesters or the like, by themselves or in combination with other monomers may also find use. The polymers may be addition or condensation polymers, particularly condensation polymers. The polymers may be cross-linked or non-cross-linked, usually not more than lightly cross-linked, generally less than 5%, usually less than 1%. For the most part, besides carbon and hydrogen, the polymers will include oxygen and nitrogen, particularly oxygen. The oxygen may be present as oxy, e.g. hydroxy or ether, carbonyl, e.g. non-oxo-carbonyl, such as carboxylic acid ester, and the like. The nitrogen may be present as amide, cyano and amino. The polymers set forth in Heller (1), supra, may find use, and that disclosure is specifically incorporated herein by reference.

Of particular interest are polymers of hydroxylaliphatic carboxylic acids, either homo- or copolymers, and polysaccharides. Included among the polyesters of interest are polymers of D-lactic acid, L-lactic acid, racemic lactic acid, glycolic acid, polycaprolactone, and combinations thereof. By employing the L-lactate, a slowly eroding polymer is achieved, while erosion is substantially enhanced with the lactate racemate.

Among the polysaccharides will be calcium alginate, and functionalized celluloses, particularly carboxymethylcellulose esters characterized by being water insoluble, a molecular weight of about 5 kD to 500 kD, etc. Other polymers of interest include polyvinyl alcohol, esters and ethers, which are biocompatible and may be biodegradable. For the most part, characteristics of the polymers will include biocompatibility, compatibility with the drug, ease of encapsulation, a half-life in the physiological environment of at least 6 hrs, preferably greater than one day, no significant enhancement of the viscosity of the vitreous, water insoluble, and the like.

For lipid encapsulating agents, the drug can be incorporated into the lumen of the vesicle which is relatively leakproof to the drug. The nature of the liposome may be widely varied, various lipids being employed for the formation of the liposome. Proteins or other non-lipid compounds may be bound to the liposome membrane which may affect the nature of the liposome. In the absence of proteinaceous compounds, acidic phospholipids will desirably be present in at least minor amounts, while in the presence of proteinaceous materials, the liposome will desirably be substantially neutral.

Among lipids which may be employed for preparation of the liposomes are phosphatidyl compounds, such as phosphatidyl choline (PC), phosphatidyl serine (PS), and phosphatidyl ethanolamine (PE); sphingolipids; cerebrosides; gangliosides; steroids, e.g. cholesterol; etc. Desirably, the liposomes will have from about 10 to 50 mole per cent steroid, with the remainder primarily being aliphatic acids and esters of both organic and inorganic acids. Small amounts of other types of lipid material may be present, generally less than about 10 mole percent, usually less than about 5 mole percent.

The biodegradable polymers which form the microencapsulated particles will desirably be subject to enzymatic or hydrolytic instability. Water soluble polymers may be cross-linked with hydrolytic or biodegradable unstable cross-links to provide useful water insoluble polymers. The degree of stability can be varied widely, depending upon the choice of monomer, whether a homopolymer or copolymer is employed, employing mixtures of polymers, where the polymers may be employed as varying layers or mixed.

By employing a biodegradable polymer, particularly one where the biodegradation is relatively slow, the

rate of release of the drug will be primarily diffusion controlled, depending upon the surrounding membrane or monolithic polymer structure, rather than breakdown of the particle. For the most part, the selected particles will have lifetimes at least equal to the desired period of administration, preferably at least twice the desired period of administration, and may have lifetimes of 5 to 10 times the desired period of administration. The period of administration will usually be at least 3 days, more usually at least 7 days, generally at least about 15 days and may be 20 days or more.

The particles may be substantially homogeneous as to composition and physical characteristics or heterogeneous. Thus, particles can be prepared where the center may be of one material and the surface have one or more layers of the same or different composition, where the layers may be cross-linked, of different molecular weight, different density or porosity, or the like. For example, the center could be a polylactate coated with a polylactate-polyglycolate copolymer, so as to enhance the rate of initial degradation. Most ratios of lactate to glycolate employed will be in the range of about 1:0-1. Alternatively, the center could be polyvinyl alcohol coated with polylactate, so that on degradation of the polylactate the center would dissolve and be rapidly washed out of the eye.

Any pharmacologically active agent for which sustained release is desirable may be employed. Desirably, the drug will be sufficiently soluble in the vitreous to be presented at a pharmacologically effective dose. Pharmacologic agents which may find use may be found in U.S. Patent Nos. 4,474,451, columns 4-6 and 4,327,725, columns 7-8, which disclosures are incorporated herein by reference.

Drugs of particular interest include hydrocortisone (5-20mcg/l as plasma level), gentamycin (6-10mcg/ml in serum), 5-fluorouracil (~30mg/kg body weight in serum, sorbinil, IL-2, TNF, Phakan-a (a component of glutathione), thiola-thiopronin, Bendazac, acetylsalicylic acid, trifluorothymidine, interferon (α , β and γ), immune modulators, e.g. lymphokines, monokines, and growth factors, etc.

Other drugs of interest include anti-glaucoma drugs, such as the beta-blockers: timolol maleate, betaxolol and metipranolol; mitotics: pilocarpine, acetylcholine chloride, isofluorophate, demacarium bromide, echothiophate iodide, phospholine iodide, carbachol, and physostigmine; epinephrine and salts, such as dipivefrin hydrochloride; and dichlorphenamide, acetazolamide and methazolamide; anti-cataract and anti-diabetic retinopathy drugs, such as aldose reductase inhibitors: tolrestat, lisinopril, enalapril, and statil; thiol cross-linking drugs other than those considered previously; anti-cancer drugs, such as retinoic acid, methotrexate, adriamycin, bleomycin, triamcinolone, mitomycin, cis-platinum, vincristine, vinblastine, actinomycin-D, ara-c, bisantrene, CCNU, activated cytoxan, DTIC, HMM, melphalan, mithramycin, procarbazine, VM26, VP16, and tamoxifen; immune modulators, other than those indicated previously; anti-clotting agents, such as tissue plasminogen activator, urokinase, and streptokinase; anti-tissue damage agents, such as superoxide dismutase; proteins and nucleic acids, such as mono- and polyclonal antibodies, enzymes, protein hormones and genes, gene fragments and plasmids; steroids, particularly anti-inflammatory or anti-fibrous drugs, such as cortisone, hydrocortisone, prednisolone, prednisone, dexamethasone, progesterone-like compounds, medrysone (HMS) and fluorometholone; non-steroidal anti-inflammatory drugs, such as ketolac tromethamine, diclofenac sodium and suprofen; antibiotics, such as loridine (cephaloridine), chloramphenicol, clindamycin, amikacin, tobramycin, methicillin, lincomycin, oxycillin, penicillin, amphotericin B, polymyxin B, cephalosporin family, ampicillin, bacitracin, carbenicillin, cephalothin, colistin, erythromycin, streptomycin, neomycin, sulfacetamide, vancomycin, silver nitrate, sulfisoxazole diolamine, and tetracycline; other anti-pathogens, including anti-viral agents, such as idoxuridine, trifluorouridine, vidarabine (adenine arabinoside), acyclovir (acycloguanosine), pyrimethamine, trisulfapyrimidine-2, clindamycin, nystatin, flucytosine, natamycin, miconazole and piperazine derivatives, e.g. diethylcarbamazine; cycloplegic and mydriatic agents, such as atropine, cyclogel, scopolamine, homatrophine and mydriacyl.

Other agents include anticholinergics, anticoagulants, antifibrinolytic agents, antihistamines, antimalarials, antitoxins, chelating agents, hormones, immunosuppressives, thrombolytic agents, vitamins, salts, desensitizing agents, prostaglandins, amino acids, metabolites and antiallergenics.

The amount of drug employed in the capsule will vary widely depending on the effective dosage required and rate of release. Usually the drug will be from about 1 to 80, more usually 20 to 40 weight percent of the microcapsule.

Other agents may be employed in the formulation for a variety of purposes. In addition to the drug agent, buffering agents and preservatives may be employed. The water soluble preservatives include sodium bisulfite, sodium thiosulfate, ascorbate, benzalkonium chloride, chlorobutanol, thimerosal, phenylmercuric borate, parabens, benzyl alcohol and phenylethanol. These agents may be present in amounts of from 0.001 to 5% by weight and preferably 0.01 to 2%. Suitable water soluble buffering agents are alkali or alkaline earth, carbonates, phosphates, bicarbonates, citrates, borates, acetates, succinates and the like, such as sodium phosphate, citrate, borate, acetate, bicarbonate and carbonate. These agents may be present in amounts sufficient to maintain a pH of the system of between 2 to 9 and preferably 4 to 8. As such the buffering agent may be as

much as 5% on a weight to weight basis of the total composition.

The particles may be of a narrow or broad range in size, normally not exceeding 300 μM , so as to be capable of being administered with an 18 gauge needle. Usually, the particle range will not differ by greater than about 200% of the average particle size, more usually not greater than about 100%. The average particle size will usually be in the range of 5 μM to 2 mM, more usually in the range of 10 μM to 1 mM. In some instances the particles will be selected to have an average diameter in the range of 1-2 mM to provide large depots, while in other instances the particles will have average diameters in the range of about 25-500 μM , to provide smaller depots. The size of the particle can be used to control the rate of release, period of treatment and drug concentration in the eye. In some situations mixtures of particles may be employed employing the same or different pharmacological agent. In this way in a single administration a course of drug treatment may be achieved, where the pattern of release may be greatly varied.

Various techniques may be employed to produce the encapsulated drugs. Useful techniques include solvent-evaporation methods, phase separation methods, interfacial methods and the like.

In preparing the polymeric encapsulated drugs, for the most part solvent-evaporation methods will be employed. Towards this end, the preformed rate controlling polymer is dissolved in a volatile substantially water-immiscible solvent, such as chloroform, methylene chloride, or benzene. Sometimes, the water immiscible solvent will be modified with a small amount of a water-miscible organic cosolvent, particularly an oxygenated solvent, such as acetone, methanol, ethanol, etc. Usually, the water-miscible organic cosolvent will be less than about 40 vol %, usually less than about 25 vol %. The drug may then be added to the polymer-solvent solution. Depending upon the nature of the drug, one may have the drug dispersed in the viscous polymer-solvent mixture or a solid dispersion of drug particles, where the drug will have been pulverized to obtain a fine powder, usually a microfine powder particularly of a size of less than about 1 mM, usually less than about 0.5 mM, and may be about 0.5 μM or smaller.

The amount of polymer employed in the medium will vary with the size of the particle desired, whether additional coatings will be added, the viscosity of the solution, the solubility of the polymer and the like. Usually, the concentration of polymer will be in the range of 10 to 80 weight percent. The ratio of drug of polymer will vary with the desired rate of release, the amount of drug generally varying in the range of 1 to 80 weight percent of the polymer.

The dispersion or solution obtained above is added to a rapidly stirred aqueous solution comprising water and a dispersing agent, which may be a protective colloid. Of particular interest as macromolecular dispersing agents are agents such as poly(vinyl alcohol) (1-5%) or non-ionic detergents, such as Span detergents.

The volume of the organic phase will be smaller than the aqueous phase, generally being in a volume ratio of from about 1:1 to 10³ of organic to aqueous phase, and an oil-in-water emulsion is produced. The rate of stirring is selected to produce the appropriate droplet size and stirring is continued throughout the next step.

In the third step, the microencapsulation vessel is closed and a mild vacuum is applied to the system to evaporate the volatile organic solvent. The solvent should be evaporated slowly, since too rapid evaporation results in bubbles and blow holes formed in the microcapsule walls. The rate of evaporation may be determined empirically, using the experience reported in the literature. Usually the vacuum will be in the range of about 3 to 10 mm Hg. After evaporation has been completed, the resulting microcapsules are centrifuged, washed completely with water, collected, e.g., filtration, and drained. Usually, the microcapsules will then be subdivided with sieves to isolate particles of a size range of the desired diameter.

The process may be carried out conveniently at room temperature, but cooling or heating may be employed in specific situations to optimize the process. The ratio of drug to polymer is adjusted to produce optimized compositions, since the final product will normally result in the initial ratio. By manipulating the initial bulk viscosity of the drug-polymer-solvent mixture and of the aqueous dispersing medium, along with the stir rate, production of microcapsules with the desired size may be optimized. Moreover, the composition of dissolved organic solvent and the rate of solvent evaporation can be tested to produce microcapsules with larger or smaller crystals of drug in the microcapsules. For polymers which are hydrolytically sensitive, the microcapsules should not be exposed to the aqueous dispersing medium for excessively long periods during the solvent-evaporation step.

The particle size distribution of each batch of microcapsules will be relatively narrow. However, when desired, the size-fractions may be further refined by a physical separation process such as dry or wet sieving.

In order to define the potential drug-release behavior of the microcapsules *in vivo*, a weighed sample of microcapsules may be added to a measured volume of a solution containing four parts of weight of ethanol and six parts of weight of deionized water. The mixture is maintained at 37°C and stirred slowly to maintain the microcapsules suspended. The appearance of the dissolved drug as a function of time may be followed spectrophotometrically until the absorbance becomes constant or until greater than 90% of the drug has been released. The drug concentration after 1 h in the medium is indicative of the amount of free unencapsulated

drug in the dose, while the time required for 90% drug to be released is related to the expected duration of action of the dose in vivo. As a general rule, one day of drug release in vitro is approximately equal to 35 days of release in vivo. While release may not be uniform, normally the release will be free of larger fluctuations from some average value which allows for a relatively uniform release.

When employing a liposome encapsulated drug the encapsulating lipid bilayer membrane may be prepared in a variety of ways. In general, the literature provides a variety of methods for liposome formation and for linking compounds to a lipid group any of which may be utilized. For the preparation of liposomes, see, in particular, Szoka and Papahadjopoulos, Proc. Natl. Acad. Sci. USA (1978) 75: 145-149.

The liposome solution will normally be isotonic with the physiological fluid in which it is to act. The pH of the solution will generally be greater than about 6 and not greater than about 9, more usually from about 6 to 8, preferably from about 6.5 to 7.5. Various buffers may be used which are physiologically acceptable, particularly phosphate, carbonate and acetate.

The concentration of the drug will vary, depending upon its physiologically effective concentration, the ability of maintain the concentration in the lumen of the liposome, the effect of the compound on the stability and impermeability of the liposome, as well as the size and number of liposomes. The drug concentration may range from about 0.01mM to about 100mM. The concentration of buffer will generally be from about 20 to about 100mM, while the concentration of salt per milliliter of solution will generally range from about 0.25 to 0.90 percent.

The microcapsules may be administered into the eye in a variety of ways, including injection, infusion, trocar, etc. Various techniques for introducing materials into the anterior and/or posterior chambers are well known, see, for example, Liu et al., 1987, supra, and references cited therein.

The following examples are offered by way of illustration and not by way of limitation.

EXPERIMENTAL

Polymeric Encapsulated Drugs

The appropriate weight of polymer is solubilized with a water immiscible organic volatile solvent, e.g. benzene, methylene chloride or chloroform. The proper amount of drug is added to the polymeric mixture to form a slurry which is mixed to substantial homogeneity. The slurry is then added dropwise to a vessel containing rapidly stirred deionized distilled water in a volume ratio of 1 : 0.5-1 x 10³ (organic slurry:water). The water is 2-5 wt % polyvinyl alcohol. The vessel is sealed and a mild vacuum applied slowly to prevent bubbles and blow holes in the microcapsules over a period of about 8-10 hrs. After evaporation of the solvent, the microcapsules are centrifuged, washed repeatedly with sterile distilled water, filtered and drained. The microcapsules are sized with sieves, dried in vacuo and may then be used directly by trocar injection for introduction into the vitreous humor of the eye. For sulfadiazine, the drug was 10 wt %, for hydrocortisone 40 wt %, and for methotrexate 25 wt % of the polymer weight. The drugs were used as microfine powders, ≤ 20 μM. The trocar injection was with a 20-gauge needle, the particles being of an average size of about 0.2 mM.

The monomer DL-lactic acid was recrystallized twice from acetone and twice from methyl ethyl ketone. The lactic acid was added to a polymerization tube, air and residual solvent removed in vacuo and the tube heated until the lactic acid melted. Catalyst (tetraphenyl tin, 0.02 wt %) was added and the tube sealed in vacuo and heated at 170-175°C for seven hours. After cooling, the tube was opened and the polymeric product dissolved in acetone, precipitated with water at room temperature and then dried in vacuo. The polymer should not be exposed to water for long periods of time, particularly during the solvent-evaporation step during microcapsule formation.

The following tables indicate the results:

Table 1

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	#	Drug ug/ml	Time Analysis	RE		LE		AC	PC
				wks	mos	AC	PC		
10	1*	sulfa-	fluores-		0	0.0	--	0	--
		diazine	cence		1	2.0	--	0	--
					2	1.5	--	0	--
					3	1.3	--	0	--
15					4	1.5	--	0	--
					5	1.6	--	0	--
					6	1.8	--	0	--
20					7	1.4	--	0	--
					8	1.5	--	0	--
					9	1.5	--	0	--
25					10	1.4	--	0	--
					11	1.6	--	0	--
					12	1.3	--	0	--

= Animal = Rabbit
 * = Microcapsules in AC (anterior chamber)
 + = Microcapsules in PC (posterior chamber)

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The sulfadiazine containing polylactic microcapsules placed in the anterior chamber of the right eye released the drug for 12 months. There was no detection of any drug in the control left eye.

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Table 2

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#	Drug ug/ml	Time Analysis	RE		LE		AC	PC
			wks	mos	AC	PC		
10	2*	sulfa- diazine		0	0.0	--	0	--
		fluores- cence		1	0	--	0.0	--
				2	0	--	2.5	--
15				3	0	--	2.6	--
				4	0	--	2.4	--
				5	0	--	2.9	--
				6	0	--	3.0	--
20				7	0	--	2.8	--
				8	0	--	2.6	--
				9	0	--	2.6	--
25				10	0	--	2.7	--
				11	0	--	2.4	--
				12	0	--	2.3	--

= Animal = Rabbit
 * = Microcapsules in AC (anterior chamber)
 + = Microcapsules in PC (posterior chamber)

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The experiment of Table 1 was repeated, employing a higher dose level.

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Table 3

#	Drug ug/ml	Time Analysis	RE		LE		AC	PC
			wks	mos	AC	PC		
3*	sulfa- diazine	fluores- cence	0		0.0	--	--	--
			2		3.1	--	--	--
			4		2.9	--	--	--
			6		3.2	--	--	--
			8		3.0	0.0	--	--

= Animal = Rabbit

* = Microcapsules in AC (anterior chamber)

+ = Microcapsules in PC (posterior chamber)

A shorter time period was used to monitor the course of the release. The data demonstrate that the drug level had equilibrated within 2 weeks (the 2 week level was the same as the 8 week level). At 8 weeks when the animal was sacrificed, the level in the posterior chamber was found to be 0. The data demonstrate that medication placed in the anterior chamber did not migrate into the posterior chamber.

Table 4

#	Drug ug/ml	Time Analysis	RE		LE	
			wks	mos	AC	PC
4*	sulfa- diazine	fluores- cence	0		0.0	--
			2		4.2	--
			4		4.3	0

= Animal = Rabbit

* = Microcapsules in AC (anterior chamber)

+ = Microcapsules in PC (posterior chamber)

This experiment repeats the experiment above, but employs a higher dosage level.

Table 5

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#	Drug ug/ml	Analysis	Time		RE		LE	
			wks	mos	AC	PC	AC	PC
10	1+ hydrocortisone succinate	HPLC		1	<.02	1.5	0	0
	2+ hydrocortisone succinate	HPLC		2	<.02	2.0	0	0
15	3+ hydrocortisone succinate	HPLC		3	<.02	2.3	0	0
	4+ hydrocortisone succinate	HPLC		4	<.02	1.0	0	0
20	5+ hydrocortisone succinate	HPLC		5	<.02	1.5	0	0
	6+ hydrocortisone succinate	HPLC		6	<.02	1.25	0	0
25	7+ hydrocortisone succinate	HPLC		7	<.02	4.1	0	0
	8+ hydrocortisone succinate	HPLC		8	<.02	2.5	0	0
30	9+ hydrocortisone succinate	HPLC		9	<.02	1.5	0	0
35	10+ hydrocortisone succinate	HPLC		10	<.02	2.4	0	0

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= Animal = Rabbit

* = Microcapsules in AC (anterior chamber)

+ = Microcapsules in PC (posterior chamber)

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Ten different animals were employed with hydrocortisone succinate as a drug incorporated into polylactic acid. The same amount of drug/polymer was placed into the right eyes of each of the animals. One animal was sacrificed at the end of each of the months indicated to generate the data. The results demonstrate the following:

1) drug placed in the posterior chamber of the eye did not migrate into the anterior chamber in detectable amounts; 2) drug was still being released at 10 months at roughly the equilibration level; 3) the left eyes which were not medicated showed no drug.

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Table 6

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#	Drug ug/ml	Analysis	Time		RE		LE	
			wks	mos	AC	PC	AC	PC
10	1+	MTX		1	--	--	<.01	1.0
	2+	MTX		2	--	--	<.01	0.9
	3+	MTX		3	--	--	<.01	1.1
	4+	MTX		4	--	--	<.01	1.0
15	5+	MTX		5	--	--	<.01	1.2
	6+	MTX		6	--	--	<.01	0.8
	7+	MTX		7	--	--	<.01	0.7

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= Animal = Rabbit
 * = Microcapsules in AC (anterior chamber)
 + = Microcapsules in PC (posterior chamber)

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Methotrexate was incorporated into polylactic acid and the microcapsules placed in the posterior chamber of the left eye. Drug did not appear to migrate to the anterior chamber. Drug was still being released at 7 months.

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Lipid Encapsulated Drugs

Preparation of liposomes:

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Doxorubicin was incorporated into liposomes by using 39.35 μ M of the drug in methanol with 19.65 μ M cardiolipin. The mixture was dried through evaporation under nitrogen. Added to the dried mixture were 100 μ M phosphatidyl choline, 68.4 μ M cholesterol, and 38.9 μ M steraylamine. The latter was mixed and dried under nitrogen. The mixture was hydrated with 10 ml 0.01 M phosphate buffer with 0.85% NaCl, pH 7.4. After a swelling time of 30 minutes the liposomes were stirred for 15 minutes, followed by sonication under nitrogen in a fixed-temperature bath at 37°C for 90 minutes. The untrapped doxorubicin was separated from liposomal-encapsulated drug by extensive dialysis against 0.01 M phosphate buffer with 0.85% NaCl, pH 7.4, at 4°C over 24 hours with several changes of buffer solution. The entrapment of doxorubicin in cardiolipin liposomes was determined by fluorescence. The size of the liposomes used ranged from 900 to 1100 angstrom units.

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I. Injection of doxorubicin into the anterior chamber (AC):

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1. 50 μ g/0.1 ml doxorubicin was injected into the AC of 10 New Zealand white rabbits. The AC was tapped for doxorubicin assay.

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2. 50 μ g/0.10 ml doxorubicin was injected into the right and left AC in each of two rabbits. The contralateral eye was given 0.10 ml of normal saline. The two animals were observed for two weeks for ocular toxicity. The results are given in Table 7.

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Table 7

Residual aqueous humor doxorubicin after AC injection of 50 ug of the unencapsulated drug into the right eye. The left eye served as control.

<u>Time (hours)</u>	<u>Sample</u>	<u>Doxorubicin (ug/ml)</u>	<u>Mean \pm SD</u>	<u>Control</u>
0	1	23.5	22.5 \pm 1.41	0
	2	21.5		
2	3	0.125	0.135 \pm 0.01	0
	4	0.145		
4	5	0.075	0.0925 \pm 0.02	0
	6	0.11		
8	7	0.025	0.030 \pm 0.01	0
	8	0.035		
16	9	<0.005	<0.005	0
	10	<0.005		

The mean half-life of doxorubicin in the AC is approximately 1 hour. Unencapsulated doxorubicin causes ocular inflammation and corneal edema within 2-3 days after AC injection. The control saline injected eyes were found to be normal on gross examination and slit-lamp biomicroscopy.

II. Injection of liposome-encapsulated doxorubicin into AC:

1. 50 μ g/0.10 ml of liposome-encapsulated doxorubicin was injected into the AC of one eye of 28 rabbits. The contralateral uninjected eye served as control.
 2. 50 μ g of doxorubicin in liposome was injected into one eye of each of two rabbits and observed for 3 weeks. The contralateral eye received 0.10 ml normal saline.
- Table 8 gives the results of these experiments.

Table 8

Residual aqueous humor doxorubicin after AC injection of 50 ug of the encapsulated drug into the left eye. The right eye served as control.

<u>Time (days)</u>	<u>Sample</u>	<u>Doxorubicin (ug/ml)</u>	<u>Mean \pm SD</u>	<u>Control</u>
0	1	40.14	39.40 \pm 2.78	0
	2	42.35		
	3	35.67		
	4	39.46		
1	5	8.10	5.90 \pm 2.14	0
	6	4.35		
	7	7.35		
	8	3.79		
2	9	2.25	2.40 \pm 0.56	0
	10	2.90		
	11	1.67		
	12	2.77		
3	13	1.53	1.22 \pm 0.22	0
	14	1.15		
	15	0.87		
	16	1.32		
4	17	0.96	0.77 \pm 0.18	0
	18	0.67		
	19	0.57		
	20	0.88		
8	21	0.19	0.19 \pm 0.06	0
	22	0.12		
	23	0.26		
	24	0.19		

Table 8 continued

<u>Time</u> <u>(days)</u>	<u>Sample</u>	<u>Doxorubicin</u> <u>(ug/ml)</u>	<u>Mean</u> \pm <u>SD</u>	<u>Control</u>
16	25	<00.005	<0.005	0
	26	<0.005		
	27	0.01		
	28	<0.005		

Detectable encapsulated doxorubicin could be found up to 2 weeks in the anterior chamber (AC). Significant amounts were present up to 8 days post AC injection. Clinically the eye tolerated the encapsulated form very well with little to no signs of inflammation and no corneal edema.

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In one of the two animals injected for clinical observation, small amounts of liposomes could be seen in the interior anterior chamber. The eye was clinically quiet.

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III. Injection of doxorubicin into the posterior chamber (PC):

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1. 50 μ g/0.1 ml doxorubicin was injected into the posterior chamber (PC) of each of 10 rabbits. The contralateral eye served as control.
 2. 50 μ g/0.10 ml of doxorubicin was injected into one eye of two animals for clinical observation for one week. Saline control was injected into the contralateral eye.
- The results of doxorubicin injection into the PC are shown in Table 9.

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Table 9

Residual vitreous doxorubicin after injection of 50 μ g of the drug into the PC of the right eye. The left eye served as control.

<u>Time (hours)</u>	<u>Sample</u>	<u>Doxorubicin (μg/ml)</u>	<u>Mean \pm SD</u>	<u>Control</u>
0	1	8.40	8.55 \pm 0.24	0
	2	8.74		
2	3	6.54	5.59 \pm 1.34	0
	4	4.65		
4	5	4.3	3.85 \pm 0.64	0
	6	3.4		
8	7	0.98	0.86 \pm 0.16	0
		0.75		
16	9	0.26	0.24 \pm 0.04	0
	10	0.21		

The half-life of free doxorubicin is approximately 3 hours. Clinically the PC injection of doxorubicin was well tolerated. No evidence of toxicity was noted.

IV. Injection of encapsulated doxorubicin into the PC.

1. 50 μ g/0.1 ml of encapsulated-liposome doxorubicin was injected into the right PC of 28 rabbits. The left eye of the rabbits served as controls.

2. Empty liposomes (saline encapsulated and prepared in the same proportions as with doxorubicin incorporation) were injected into the vitreous of 4 eyes in the following volume: 0.0125 ml, 0.025ml, 0.05ml and 0.10ml. The animals were observed, as controls, up to four months.

Table 10

Residual vitreous doxorubicin after injection of encapsulate doxorubicin in the PC of the right eye. The left eye served as control.

Time (days)	Sample	Doxorubicin (ug/ml)	Mean \pm SD	Control
0	1	8.56	7.09 \pm 1.41	0
	2	7.56		
	3	7.05		
	4	5.19		
1	5	4.10	3.51 \pm 0.48	0
	6	3.55		
	7	2.94		
	8	3.45		
2	9	1.92	1.82 \pm 0.43	0
	10	1.62		
	11	1.38		
	12	2.38		
3	13	1.98	2.13 \pm 0.27	0
	14	2.25		
	15	1.85		
	16	2.45		
4	17	1.76	2.33 \pm 0.20	0
	18	2.13		
	19	1.69		
	20	1.98		
8	21	0.98	1.72 \pm 0.86	0
	22	1.30		
	23	2.94		
	24	1.65		
14	25	2.91	2.02 \pm 1.01	0
	26	2.89		
	27	1.05		
	28	1.25		

The liposome-encapsulated doxorubicin was observed in the vitreous (PC) as a localized, dense opacity immediately following injection. What appears as an inflammatory process was seen during the first 7-10 days. The margin of the vitreous capacity gradually blurred and the localized dense opacity began to decrease and fade. Opacity of much of the vitreous occurred in all cases and clouded the visualization of the fundus up to 14 days of the experiment. This clouding of the vitreous did not correlate with the clearance of the doxorubicin which cleared within the first two days of the experiment. Residual levels of doxorubicin became constant and was maintained at a significant level for up to two weeks. Perhaps, the residual vitreous doxorubicin is bonded with liposomes resulting in minimal clearance from the vitreous.

The vitreous opacity was seen in all the eyes receiving empty liposomes up to the entire four months of observation. The opacity is not drug related but was most likely due to interaction of the phospholipids of the liposomes and the vitreous gel. The vitreous induced clouding by liposomes did not diminish after the first week and appears to be permanent, at least to the extend of our observations.

It is evident from the above results that microcapsules can find effective use internal to the eye chamber for treatment of a wide variety of conditions. The microcapsules provide for continuous administration of a drug over long periods of time, avoiding the need of a patient to administer drugs in much less effective ways, such as topically. In addition, treatments can be achieved by maintaining appropriately therapeutic levels of drugs in the eye, minimizing high concentrations throughout the host system which may have deleterious effects. The drug is retained in the appropriate site, since migration is not observed to other chambers or eyes. Equilibration levels are rapidly achieved and maintained for long periods of time. Furthermore, one or only a few drug administrations may be required for treatments over extended periods of time, reducing the burden on the patient for self-administration, ensuring continued controlled medication, and minimizing the interference with the activities of the patient.

Both polymeric and lipid encapsulation protect doses of pharmacological agents from being diluted or degraded in the general circulation. The agents can be entrapped in various concentrations without any modifications. Encapsulation provides concentrated doses of medication which are more effective and less toxic than free drugs. Further, the drugs in liposomes can be protected from enzymatic attack or immune recognition because liposomes are biocompatible and nontoxic, being similar to cell membranes.

Claims

1. Use of a composition for the manufacture of a medicament for treating an eye condition which composition comprises :

a pharmacologically active agent surrounded by a barrier to immediate release wherein said composition is capable of introduction into a chamber of said eye and said agent is released from said barrier at a rate to provide an effective dosage of said agent over an extended period of time.

2. Use of a composition according to claim 1, wherein said barrier is selected from pharmacologically acceptable biodegradable polymers and liposomes.

3. Use of composition for the manufacture of a medicament for treating an eye condition which composition comprises :

microencapsulated drug particles of not greater than about 300 μ (micrometers), said particles comprising said drug and a pharmacologically acceptable biodegradable polymer wherein said composition is capable of introduction into a chamber of said eye and is degradable at a rate to provide an effective dosage of said drug over an extended period of time .

4. Use of a composition according to claims 2 or 3, wherein said polymer is a condensation polymer.

5. Use of a composition according to claim 3, wherein said particle comprises a pharmacologically acceptable buffering agent.

6. Use of a composition according to claim 3, wherein said drug is at least one of a cytotoxic agent or a growth factor.

7. Use of a composition according to claim 3, wherein said composition contains an effective dosage for treatment of said eye condition wherein said dosage is sufficient to be maintained in said chamber for at least one month.

8. Use of a composition according to claim 7, wherein said particle is of an average size in the range of 10 to 250 μ (micrometers).

9. Use of a composition condition for the manufacture of a medicament for treating an eye condition which composition comprises :

liposomes enclosing in their lumen an isotonic solution comprising a pharmacologically active agent, wherein said composition is capable of introduction into a chamber of said eye and said agent is released in the eye at a rate to provide an effective dosage of said agent over an extended period of time.

10. Use of a composition according to claim 9, wherein said liposome has from about 10 to 50 mole percent cholesterol in the lipid bilayer.

Patentansprüche

1. Verwendung einer Zusammensetzung für die Herstellung eines Arzneimittels zur Behandlung eines Au-

genleidens, wobei die Zusammensetzung umfaßt:

ein pharmakologisch aktives Mittel, umgeben von einer Sperre für die unmittelbare Freisetzung, wobei die Zusammensetzung zur Einführung in eine Augenkammer geeignet ist, und das Mittel aus der Sperre in einem Ausmaß zur Bereitstellung einer wirksamen Dosis des Mittels während eines längeren Zeitraums freigesetzt wird.

2. Verwendung einer Zusammensetzung nach Anspruch 1, worin die Sperre ausgewählt ist, aus pharmakologisch brauchbaren, bioabbaubaren Polymeren und Liposomen.

3. Verwendung einer Zusammensetzung für die Herstellung eines Arzneimittels zur Behandlung eines Augenleidens, wobei die Zusammensetzung umfaßt:

mikroeingekapselte Arzneimittelteilchen von nicht größer als etwa 300 µ (Mikrometer), wobei die Teilchen das Arzneimittel in einem pharmakologisch brauchbaren, bioabbaubaren Polymer umfassen, wobei die Zusammensetzung zur Einführung in eine Augenkammer geeignet ist, und in einem Ausmaß zur Bereitstellung einer wirksamen Dosis des Arzneimittels während eines längeren Zeitraums abbaubar ist.

4. Verwendung einer Zusammensetzung nach Anspruch 2 oder 3, worin das Polymer ein Kondensationspolymer ist.

5. Verwendung einer Zusammensetzung nach Anspruch 3, worin das Teilchen ein pharmakologisch brauchbares Puffermittel umfaßt.

6. Verwendung einer Zusammensetzung nach Anspruch 3, worin das Arzneimittel mindestens ein zytotoxisches Mittel oder Wachstumsfaktor ist.

7. Verwendung einer Zusammensetzung nach Anspruch 3, worin die Zusammensetzung eine wirksame Dosis zur Behandlung des Augenleidens enthält, wobei die Dosis ausreicht, in der Kammer während mindestens einem Monat aufrechterhalten zu werden.

8. Verwendung einer Zusammensetzung nach Anspruch 7, worin das Teilchen eine durchschnittliche Größe im Bereich von 10 bis 250 µ (Mikrometer) aufweist.

9. Verwendung einer Zusammensetzung zur Herstellung eines Arzneimittels zur Behandlung eines Augenleidens, wobei die Zusammensetzung umfaßt:

Liposome, die in ihrem Lumen eine isotonische Lösung einschließen, die ein pharmakologisch aktives Mittel umfaßt, wobei die Zusammensetzung zur Einführung in eine Augenkammer geeignet ist, und das Mittel in dem Auge in einem Ausmaß zur Bereitstellung einer wirksamen Dosis des Mittels während eines längeren Zeitraums freigesetzt wird.

10. Verwendung einer Zusammensetzung nach Anspruch 9, wobei das Liposom etwa 10 bis 50 Molprozent Cholesterin in der Lipid-Doppelschicht enthält.

Revendications

1. Utilisation d'une composition pour la fabrication d'un médicament pour traiter un état de l'oeil laquelle composition comprend:

un agent pharmaceutiquement actif entouré d'un obstacle à une libération immédiate dans lequel ladite composition est susceptible d'être introduite dans une chambre dudit oeil et ledit agent est libéré à partir dudit obstacle à une vitesse fournissant une dose efficace dudit agent pendant une période de temps prolongée.

2. Utilisation d'une composition selon la revendication 1, dans laquelle ledit obstacle est choisi parmi des polymères et liposomes biodégradables acceptables pharmaceutiquement.

3. Utilisation de composition pour la fabrication d'un médicament pour traiter un état de l'oeil laquelle composition comprend:

des particules microencapsulées de médicament ne dépassant pas environ 300 µm (micromètres), lesdites particules comprenant ledit médicament et un polymère biodégradable pharmacologiquement acceptable dans lesquelles ladite composition peut être introduite dans une chambre dudit oeil et est dégradable à une vitesse fournissant une dose efficace dudit agent pendant une période de temps prolongée.

4. Utilisation d'une composition selon les revendications 2 ou 3, dans laquelle ledit polymère est un polymère de condensation.

5. Utilisation d'une composition selon la revendication 3, dans laquelle ladite particule comprend un agent tampon pharmacologiquement acceptable.

6. Utilisation d'une composition selon la revendication 3, dans laquelle ledit médicament est au moins un agent cytotoxique ou un facteur de croissance.

7. Utilisation d'une composition selon la revendication 3, dans laquelle ladite composition contient une dose efficace pour le traitement dudit état de l'oeil dans laquelle ladite dose est suffisante pour pouvoir être maintenue dans ladite chambre pendant au moins un mois.

8. Utilisation d'une composition selon la revendication 7, dans laquelle ladite particule a une taille moyenne comprise dans un intervalle allant de 10 à 250 μm (micromètres).

9. Utilisation d'une composition pour la fabrication d'un médicament pour traiter un état de l'oeil laquelle composition comprend:

5 des liposomes englobant dans leur lumière une solution isotonique comprenant un agent pharmacologiquement actif, dans lesquels ladite composition est susceptible d'être introduite dans une chambre dudit oeil et ledit agent est libéré dans l'oeil à une vitesse fournissant une dose efficace dudit agent pendant une période de temps prolongée.

10 10. Utilisation d'une composition selon la revendication 9, dans laquelle ledit liposome a entre environ 10 et 50 pourcents en mole de cholestérol dans sa bicouche lipidique.

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